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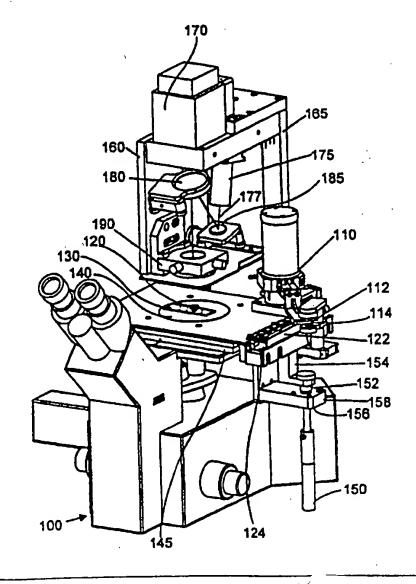
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(54) Title: SMALL DIAMETER LASER CAPTURE MICRODISSECTION

(57) Abstract

Systems and methods for small diameter laser capture microdissection are disclosed. An inverted microscope adapted for small diameter laser capture microdissection includes an illumination/laser beam delivery system with a variable position lens. A method for small diameter laser capture microdissection includes delivering energy having a pulse width of approximately 1 mS, or less, and a peak power of approximately 50 mW, or more. The systems and methods provide the advantage of permitting small regions of tissue samples, for example, under approximately 10 microns in diameter, to be microdissected.

SMALL DIAMETER LASER CAPTURE MICRODISSECTION

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15   **Field of the Invention**

The invention relates generally to the field of laser capture
microdisection (LCM). More particularly, the invention relates to inverted
microscopes that include specialized apparatus for performing LCM.
20   Specifically, a preferred implementation of the invention relates to an inverted
microscope that includes a cap handling subsystem, an illumination/laser optical
subsystem, a vacuum chuck subsystem, and a manual joystick subsystem. The
invention thus relates to inverted microscopes of the type that can be termed
laser capture microdisection inverted microscopes.

25

            Discussion of the Related Art

Diseases such as cancer have long been identified by examining tissue
biopsies to identify unusual cells. The problem has been that there has been no
30   satisfactory prior-art method to extract the cells of interest from the surrounding

tissue. Currently, investigators must attempt to manually extract, or microdissect, cells of interest either by attempting to mechanically isolate them with a manual tool or through a convoluted process of isolating and culturing the cells. Most investigators consider both approaches to be tedious, time-

5   consuming, and inefficient.

A new technique has been developed which can extract a small cluster of cells from a tissue sample in a matter of seconds. The technique is called laser capture microdissection (LCM). Laser capture microdissection is a one-step technique which integrates a standard laboratory microscope with a low-energy

10   laser and a transparent ethylene vinyl acetate polymer thermoplastic film such as is used for the plastic seal in food product packaging.

In laser capture microdissection, the operator looks through a microscope at a tissue biopsy section mounted on a standard glass histopathology slide, which typically contains groups of different types of cells.

15   A thermoplastic film is placed over and in contact with the tissue biopsy section. Upon identifying a group of cells of interest within the tissue section, the operator centers them in a target area of the microscope field and then generates a pulse from a laser such as a carbon dioxide laser having an intensity of about 50 milliwatts (mW) and a pulse duration of between about 50 to about 500

20   milliseconds (mS). The laser pulse causes localized heating of the plastic film as it passes through it, imparting to it an adhesive property. The cells then stick to the localized adhesive area of the plastic tape directly above them, whereupon the cells are immediately extracted and ready for analysis. Because of the small diameter of the laser beam, extremely small cell clusters may be microdissected

25   from a tissue section.

By taking only these target cells directly from the tissue sample, scientists can immediately analyze the gene and enzyme activity of the target cells using other research tools. Such procedures as polymerase chain reaction amplification of DNA and RNA, and enzyme recovery from the tissue sample

30   have been demonstrated. No limitations have been reported in the ability to

amplify DNA or RNA from tumor cells extracted with laser capture microdissection.

Laser capture microdissection has successfully extracted cells in all tissues in which it has been tested. These include kidney glomeruli, in situ
5   breast carcinoma, atypical ductal hyperplasia of the breast, prostatic interepithielial neoplasia, and lymphoid follicles. The direct access to cells provided by laser capture microdissection will likely lead to a revolution in the understanding of the molecular basis of cancer and other diseases, helping to lay the groundwork for earlier and more precise disease detection.

10   Another likely role for the technique is in recording the patterns of gene expression in various cell types, an emerging issue in medical research. For instance, the National Cancer Institute's Cancer Genome Anatomy Project (CGAP) is attempting to define the patterns of gene expression in normal, pre-cancerous, and malignant cells. In projects such as CGAP, laser capture
15   microdissection is a valuable tool for procuring pure cell samples from tissue samples.

The LCM technique is generally described in the recently published article: Laser Capture Microdissection, Science, Volume 274, Number 5289, Issue 8, pp 998-1001, published in 1996, the entire contents of which are
20   incorporated herein by reference. The purpose of the LCM technique is to provide a simple method for the procurement of selected human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is placed on a glass microscope slide using techniques well known in the
25   field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.

LCM employs a thermoplastic transfer film that is placed on top of the
30   tissue sample. This film is manufactured containing organic dyes that are

chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed. The film is then lifted from the tissue and the selected portion of the tissue is removed with the film.

Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electroseal Corporation of Pompton Lakes, New Jersey (type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90° C.

The thermoplastic EVA films used in LCM techniques have been doped with dyes, such as an infrared napthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that overlaps with laser emitters used to selectively melt the film. The dye is mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film manufacturing techniques. The dye concentration in the plastic is about 0.001 M.

While the films employed in LCM applications have proved satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

The organic dyes which are used to alter the absorption characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

SUMMARY OF THE INVENTION

There is a particular need for an instrument that is well suited for laser capture microdissection. There is also a particular need for an improved method of laser capture microdissection.

A first aspect of the invention is implemented in an embodiment that is
5   based on a laser capture microdissection method, comprising: providing a sample that is to undergo laser capture microdissection; positioning said sample within an optical axis of a laser capture microdissection instrument; providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface; placing said laser
10   capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdisection film; and then transferring a portion of said sample to said laser
15   capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film.

A second aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection instrument, comprising: an inverted microscope including: an illumination/laser optical subsystem; a translation
20   stage coupled to said illuminator/laser optical subsystem; a cap handling subsystem coupled to said translation stage; a vacuum chuck subsystem coupled to said translation stage; and a manual joystick subsystem coupled to said translation stage.

These, and other, aspects and objects of the invention will be better
25   appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating preferred embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the

scope of the invention without departing from the spirit thereof, and the

invention includes all such modifications.

BRIEF DESCRIPTION OF THE DRAWINGS

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A clear conception of the advantages and features constituting the

invention, and of the components and operation of model systems provided with

the invention, will become more readily apparent by referring to the exemplary,

and therefore nonlimiting, embodiments illustrated in the drawings

10   accompanying and forming a part of this specification, wherein like reference

numerals (if they occur in more than one view) designate the same elements.

Consequently, the claims are to be given the broadest interpretation that is

consistent with the specification and the drawings. It should be noted that the

features illustrated in the drawings are not necessarily drawn to scale.

15   FIG. 1 illustrates a perspective view of a laser capture microdissection

inverted microscope, representing an embodiment of the invention.

FIGS. 2A-2B illustrate orthographic views of the laser capture

microdissection (LCM) inverted microscope shown in FIG. 1.

FIG. 3 illustrates a partial cross-sectional view of an LCM inverted

20   microscope, representing an embodiment of the invention.

FIG. 4 illustrates a partial cross-sectional view of an LCM inverted

microscope, representing an embodiment of the invention.

FIG. 5 illustrates a cross-sectional view of a cap handling subassembly,

representing an embodiment of the invention.

25   FIG. 6 illustrates an elevational view of a cap handling subassembly in a

load position, representing an embodiment of the invention.

FIG. 7 illustrates a top plan view of the apparatus in the position

depicted in FIG. 6.

FIG. 8 illustrates an elevational view of a cap handling subassembly in

30   an inspect position, representing an embodiment of the invention.

FIG. 9 illustrates a top plan view of the apparatus in the position depicted in FIG. 8.

FIG. 10 illustrates an elevational view of a cap handling subassembly in an unload position, representing an embodiment of the invention.

5   FIG. 11 illustrates a top plan view of the apparatus in the position depicted in FIG. 10.

FIG. 12 illustrates a top plan view of a vacuum chuck, representing an embodiment of the invention.

FIG. 13 illustrates a cross-sectional view of a vacuum chuck,

10   representing an embodiment of the invention.

FIG. 14 illustrates a schematic diagram of a combined illumination light/laser beam delivery system, representing an embodiment of the invention.

FIG. 15 illustrates a schematic view of a combined illumination/laser beam delivery system with a diffuser in place, representing an embodiment of

15   the invention.

FIG. 16 illustrates a schematic view of a combined illumination/laser beam delivery system with a cap in place, representing an embodiment of the invention.

FIG. 17 illustrates a schematic view of an integrated cap/diffuser,

20   representing an embodiment of the invention.

FIG. 18 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention.

FIG. 19 illustrates available energy as a function of perpendicular distance from the optical axis of a 5 micron diameter laser beam and a 30

25   micron diameter laser beam, representing embodiments of the invention.

FIG. 20 illustrates a geometric plot of three isotherms within an LCM transfer film corresponding to three different laser beam pulse durations each of which delivers a different total amount of energy, representing embodiments of the invention.

FIG. 21 illustrates a geometric plot of three isotherms within an LCM transfer film corresponding to three different laser beam pulse durations each of which delivers a substantially constant total amount of energy, representing embodiments of the invention.

5   FIG. 22 illustrates a schematic isometric view of a focusing lens subassembly representing an embodiment of the invention.

FIG. 23 illustrates a schematic isometric view of the focusing lens subsystem mounted on an optics plate of an inverted microscope, representing an embodiment of the invention.

10   FIG. 24 illustrates a schematic perspective view of the apparatus shown in FIG. 23 with a focusing lens position control in a first position, representing an embodiment of the invention.

FIG. 25 illustrates another schematic perspective view of the apparatus shown in FIGS. 23-24 with the focusing lens position control in a second

15   position, representing an embodiment of the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

The invention and the various features and advantageous details thereof

20   are explained more fully with reference to the nonlimiting embodiments that are illustrated in the accompanying drawings and detailed in the following description. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the invention in detail.

The entire contents of U.S. Ser. No. 60/037,864, filed February 7, 1997

25   entitled "Laser Capture Microdissection Device," (Docket No. ARCT-002); U.S. Ser. No. 08/797,026, filed February 7, 1997; U.S. Ser. No. 08/800,882, filed February 14, 1997; U.S. Ser. No. 60/060,731, filed October 1, 1997; and U.S. Ser. No. 60/060,732, filed October 1, 1997 are hereby expressly incorporated by reference into the present application as if fully set forth herein.

Turning to FIG. 1, a perspective view of an inverted microscope 100 for laser capture microdissection (LCM) is depicted. The inverted microscope 100 includes a variety of subsystems, particularly adapted for the LCM technique which combine to provide synergistic and unexpectedly good results. In

5   alternative embodiments, the microscope does not need to be an inverted microscope.

A cap handling mechanic subassembly 110 provides structure for picking a microcentrifuge tube cap 120 from a supply 122 and placing the microcentrifuge tube cap 120 on top of a sample that is to undergo LCM. In the

10   depicted embodiment, the microcentrifuge tube cap 120 is a cylindrical symmetric plastic cap and the supply 122 includes eight of the consumables on a dovetail slide 124. In the depicted embodiment, there is a laser capture microdissection transfer film coupled to the bottom of the microcentrifuge tube cap 120. The cap handling mechanic subassembly 110 is depicted in one of

15   several possible positions wherein a working end 112 of the cap handling mechanic subassembly 110 is positioned in a vial capping station 114. The movement of the cap handling mechanic subassembly 110 will be described in more detail below.

A glass slide 130 upon which the sample to be microdissected is located

20   and upon which the microcentrifuge tube cap 120 is placed, is located in the primary optical axis of the inverted microscope 100. In alternative embodiments, the slide that supports the sample can be made of other substantially transparent materials, for example, plastics such as polycarbonate. The glass slide 130 is supported and held in place by a vacuum chuck 140. The

25   vacuum chuck 140 is a substantially flat surface that engages the glass slide 130 through a manifold (not shown) so as to hold the glass slide 130 in place while the microcentrifuge tube cap 120 is picked and placed and while a translation stage 145 is manipulated in an X-Y plane. In alternative embodiments, the translation stage can be configured so as to have the capability of being moved

30   along a Z axis.

The translation stage 145 can be manipulated using a pair of rotary controls (not shown in FIG. 1). In addition, the translation stage 145 can be manipulated using a joystick 150. The joystick 150 is connected to the translation stage 145 through a spherical mounting 152 and a bracket 154. The

5   joystick 150 includes a second spherical mounting 156 within a static bracket 158. The joystick provides simultaneous X and Y movement. Further, this simultaneous movement can be effected with in a single handed manner. The acquisition of samples is thus made quicker.

Mechanical leverage is provided by the fact that the length between the

10   spherical mounting 152 and the second spherical mounting 156 is less than the length between the second spherical mounting 156 and the bottom end of the joystick 150. This leverage ratio is not needed for multiplication of force, but for the reduction in scalar movement. This ratio should be less than 1/5, preferably approximately 1/7. This ratio can be adjusted to provide the optimal

15   resolution needed in terms of sample movement as a function of operator hand movement.

In addition, the joystick provides tactile feedback not available with electronic controls or geared linkages. The joystick 150 permits simultaneous movement of the translation stage 145 in two directions (X and Y) as a function

20   of a single vector movement of an operator's hand. This important feature provides an unexpected result in that the speed with which features to be microdissected can be positioned in the principal optical axis of the inverted microscope 100 is significantly increased.

Still referring to FIG. 1, the inverted microscope 100 includes an LCM

25   optical train 160. The LCM optical train 160 is mounted on an illumination arm 165. A white light illuminator 170 is also mounted on the illumination arm 165. White light from the illuminator 170 passes downward toward the microcentrifuge tube cap 120 through a dichroic mirror 180 and a focusing lens 190. A laser diode 175 with collimating optics emits a beam 177 that is

30   reflected by a beam steering mirror 185. After the beam 177 is reflected by the

-10-

beam steering mirror 185 it is incident upon the dichroic mirror 180. The dichroic mirror 180 is a dichroic that reflects the beam 170 downward through the focusing lens 190 toward the microcentrifuge tube cap 120. Simultaneously, the dichroic mirror 180 allows white light from the illuminator 170 to pass

5   directly down through the focusing lens 190 toward the microcentrifuge tube cap 120. Thus, the beam 177 and the white light illumination are superimposed. The focusing lens 190 also adjusts the beam spot size.

Turning now to FIGS. 2A-2B, two orthographic views of the apparatus depicted in FIG. 1 are illustrated. A white light illumination path 210 and a

10   laser beam path 220 can be seen in both FIGS. 2A and 2B. It can be appreciated from FIG. 2A that both of the paths include delivery of optical information to an image acquisition system 230. Similarly, it can be appreciated from FIG. 2B that the illumination beam path includes delivery of optical information to a binocular set 240. In alternative embodiments, the eyepiece assembly (i.e.,

15   ocular) can include a monocular.

Turning to FIG. 3, a block schematic diagram of an optical train according to the invention is depicted. A laser beam path 310 begins at a film activation laser 320. The laser beam path 310 is then reflected by a mirror 330. The laser beam path 310 is then reflected by a dichroic mirror 340. The laser

20   beam path 310 is then focused by a lens 350. The lens 350 can optionally be associated with structure for changing the beam diameter such as, for example, a variable aperture. The laser beam path 310 then passes downward toward the microcentrifuge tube cap 120. The laser beam path 310 then passes through an objective lens 360 and is then reflected. A cut-off filter 390 is installed in the

25   ocular 370. The cut-off filter 390 can reflect and/or absorb the energy from the laser beam.

The position of the laser beam path 310 with respect to the portion of the sample that is to be acquired by the microcentrifuge tube cap 120 can be seen by an operator via the image acquisition system 230 (not shown in FIG. 3), which

30   can include a camera. In idle mode, the laser beam path 310 provides a visible

low amplitude signal that can be detected via the acquisition system 230. In pulse mode, the laser beam path 310 delivers energy to the microcentrifuge tube cap 120 and the optical characteristics of the cut-off filter 390 attenuate the laser beam path 310 sufficiently so that substantially none of the energy from the

5   laser beam exits through ocular 370.

Suitable laser pulse widths are from 0 to approximately 1 second, preferably from 0 to approximately 100 milliseconds, more preferably approximately 50 milliseconds. In a preferred embodiment the wavelength of the laser is 810 nannometers. In a preferred embodiment the spot size of the

10   laser at the EVA material located on microcentrifuge tube cap 120 is variable from 0.1 to 100 microns, preferably from 1 to 60 microns, more preferably from 5 to 30 microns. These ranges are relatively preferred when designing the optical subsystem. From the standpoint of the clinical operator, the widest spot size range is the most versatile. A lower end point in the spot size range on the

15   order of 5 microns is useful for transferring single cells.

Suitable lasers can be selected from a wide power range. For example, a 100 watt laser can be used. On the other hand, a 50 mW laser can be used. The laser can be connected to the rest of the optical subsystem with a fiber optical coupling. Smaller spot sizes are obtainable using diffraction limited laser

20   diodes and/or single mode fiber optics. Single mode fiber allows a diffraction limited beam.

While the laser diode can be run in a standard mode such as TEM_{00}, other intensity profiles can be used for different types of applications. Further, the beam diameter could be changed with a stepped lens instead of lens 350.

25   Changing the beam diameter permits the size of the portion of the sample that is acquired to be adjusted. Given a tightly focused initial condition, the beam size can be increased by defocusing. Given a defocused initial condition, the beam size can be decreased by focusing. The change in focus can be in fixed amounts. The change in focus can be obtained by means of indents

30   on a movable lens mounting and/or by means of optical glass steps. In any

event, increasing/decreasing the optical path length is the effect that is needed to alter the focus of the beam, thereby altering the spot size. For example, inserting a stepped glass prism 380 into the beam so the beam strikes one step tread will change the optical path length and alter the spot size.

5   Turning now to FIG. 4, a schematic block diagram of another embodiment of an instrument according to the invention is depicted. In this embodiment, a light source 410 (e.g., fluorescence laser), emits a specific wavelength or wavelength range. The specific wavelength or wavelength range of a beam 420 emitted by the light source 410 is chosen, or filtered, to excite a

10   fluorescent system (e.g., chemical markers and optical filtering techniques that are known in the industry) that is incorporated in or applied to the sample to be microdissected. The frequency of a beam 420 emitted by the fluorescence laser 410 can be tuned. The sample includes at least one member selected from the group consisting of chromophores and fluorescent dyes (synthetic or organic),

15   and, the process of operating the instrument includes identifying at least a portion of said sample with light that excites the at least one member, before the step of transferring said portion of said sample to said laser capture microdissection transfer film.

Still referring to FIG. 4, the beam 420 is reflected by a mirror 430. The

20   beam 420 is then reflected by the dichroic mirror 340. In this way the beam 420 can be made coincident with both the laser beam path 310 and the white light from illuminator 170. It should be noted that the beam 420 and the laser beam path 310 are shown in a spaced-apart configuration for clarity only. The beam 420 and the laser beam path 310 can be coaxial. Fluorescence emitted by the

25   sample beneath the microcentrifuge tube cap 120 then travels through the objective lens 360 to be viewed by the operator through ocular 370.

Turning now to FIG. 5, a cross-sectional view of the cap handling mechanic subassembly 110 is depicted. The cap handling mechanic subassembly 110 includes a dampener 510. The dampener 510 is a structure for

30   damping vertical motion of the cap handling mechanic subassembly 110. The

dampener 510 is adapted to lower the microcentrifuge tube cap 120 down towards the translation stage in a reproducible manner. The dampener 510 can be an air dampener (e.g., pneumatic tube) or liquid dampener (e.g., hydraulic tube) or any other dynamic structure capable or retarding the vertical motion of

5   the subassembly 110 so as not to generate an impulse. As the microcentrifuge tube cap 120 contacts the slide on which the sample rests (not shown), the working end 112 of an arm 520 that is coupled to the dampener 510 continues downward at a reproducible rate. Therefore, the top of the microcentrifuge tube cap 120 rises relative to the bottom of a weight 530. It can be appreciated that

10   the cap 120 contacts the slide, before the weight 530 contacts the cap 120. In this way, the microcentrifuge tube cap 120 undergoes a self-leveling step before it is contacted and pressed against the slide by weight 530. As the weight 530 contacts the microcentrifuge tube cap 120 the working end 112 of arm 520 continues along its downward path. Therefore, the application of the weight

15   530 to microcentrifuge tube cap 120 is also a self-leveling step. By controlling the mass of weight 530, the force per unit area between the bottom of the microcentrifuge tube cap 120 and the slide can be controlled. After the sample on the slide has undergone LCM, the arm 520 can be raised. By raising the arm, the weight 530 is first picked off the microcentrifuge tube cap 120 and then the

20   microcentrifuge tube cap 120 is picked up off of the slide. The dampener within the mechanism acts as a dash pot to control the velocity of the pickup arm.

        The position of the translation stage is independent relative to the position of the cap handling mechanic subassembly 110. These relative positions can be controlled by the pair of rotary controls 147. It is to be noted

25   that the pair of rotary controls 147 are depicted with their axes parallel to the axis of the microcentrifuge tube cap 120 in FIG. 5. However, the pair of rotary controls 147 can be configured in any orientation through the use of mechanical linkages such as gears.

        Turning now to FIG. 6, the cap handling mechanic subassembly 110 is

30   depicted in a load position. In the load position, the working end 112 of the arm

520 is located directly over the dovetail slide 124. In this position, the working
end 112 grasps a microcentrifuge tube cap 120. After grasping the
microcentrifuge tube cap 120, the arm 520 is raised, thereby picking the
microcentrifuge tube cap 120 up.

5   Turning now to FIG. 7, a top plan view of the cap handling mechanic
subassembly 110 in the load position can be seen. Before the arm 520 is swung
into the load position, a fresh microcentrifuge tube cap is located beneath the
axis of the working end 112. After the arm 520 is swung clockwise toward the
vacuum chuck 140, the caps on dovetail slide 124 will be advanced so as to
10   position a fresh microcentrifuge tube cap in place for the next cycle.

Turning now to FIG. 8, the cap handling mechanic subassembly 110 is
depicted in an inspect position. When positioned in the inspect position, the
working end 112 of the arm 520 is located coincident with the principal optical
axis of the instrument. This is the position in which the arm 520 is lowered to
15   permit first the self-leveling of the microcentrifuge tube cap 120 and then the
self-leveling of the weight 530 on top of the microcentrifuge tube cap 120.
After LCM, the arm 520 is raised in this position to put the weight 530 off the
microcentrifuge tube cap 120 and then the microcentrifuge tube cap 120 off the
slide (not shown).

20   The weight 530 is a free floating weight so that when it is set on top of
the cap, the cap and weight are free to settle. The free floating weight permits
the even application of pressure. For example, a weight of 30 grams can be
used in the case where the total surface area of the laser capture microdissection
transfer film is approximately 0.26 square inches.

25   Referring now to FIG. 9, a top plan view of the cap handling mechanic
subassembly 110 in the inspect position is depicted. It can be appreciated from
this view that the working end 112 of the arm 520 is located above the glass
slide 130.

Turning now to FIG. 10, the cap handling mechanic subassembly 110 is
30   depicted in an unload position. In the unload position, the working end 112 of

the arm 520 and the cap 120 (aka consumable) with the LCM attached tissue are all located above the vial capping station 114. After being positioned on axis with the vial capping station 114, the microcentrifuge tube cap 120 is lowered directly down onto, and into, an analysis container 1000. After the

5   microcentrifuge tube cap 120 is inserted into the analysis container 1000, the working end 112 of the arm 520 is raised up. The working end 112 of the arm 520 is then rotated in a clockwise direction until it is above a fresh consumable (corresponding to the position depicted in FIGS. 6-7).

Turning now to FIG. 11, a top plan view of the cap handling mechanic

10   subassembly 110 in the unload position is depicted. In this position, the arm 520 is positioned away from the vacuum chuck 140. The analysis container 1000 (not visible in FIG. 11) is pushed upward so as to engage the microcentrifuge tube cap 120 (not visible in FIG. 11). The resultant sealed analysis container 1000 is then allowed to free fall back into a supporting

15   bracket 1010 (see FIG. 10). The sealed analysis container 1000 together with the microcentrifuge tube cap 120 can then be taken from the bracket 1010 either manually or automatically.

Turning now to FIG. 12, a top plan view of the vacuum chuck 140 is depicted. A top surface 1210 of the vacuum chuck 140 includes a first manifold

20   hole 1020 and a second manifold hole 1030. In alternative embodiments, there can be any number of manifold holes. The vacuum chuck 140 includes a beam path hole 1040. When the instrument is in operation, the glass slide (not shown), or other sample holder, is placed over the beam path hole 1040 and the manifold holes 1020-1030. After the glass slide is placed in position, a vacuum

25   is pulled through a manifold connected to the holes 1020-1030, thereby holding the glass slide in position over the beam path hole 1040. Although a medium or even a high vacuum can be applied, a low vacuum is sufficient to hold the glass slide in place during the LCM process. A sufficient vacuum can even be generated with an inexpensive aquarium pump run in reverse.

The holding force exerted on the glass slide 130 is a function of the applied vacuum, the size and shape of the manifold holes 1020-1030 and the spacing between the top surface of the translation stage and the bottom surface of the glass slide 130. The spacing between the translation stage and the glass slide 130 is a function of the flatness of the surfaces and the elasticity of the corresponding structures.

The level of vacuum is adjusted so that the glass slide 130, or other sample carrier, can be translated with regard to the translation stage. This translation capability is present when the vacuum is off and when the vacuum is on.

There is some leakage around the perimeter of the glass slide 130 which modulates the force holding the glass slide 130 in place. Accordingly, a moderate force (e.g., 5 pounds) applied to the edge of the glass slide is sufficient to cause movement of the glass slide 130 with regard to the translation stage when the vacuum is engaged.

Turning now to FIG. 13, a cross section of the vacuum chuck is depicted with a glass slide 130 in place. The vacuum that holds the glass slide 130 in place is pulled through conduit 1320. The conduit 1320 is connected to a circular manifold 1310. The circular manifold 1310 is coupled with the manifold holes 1020-1030.

It can be appreciated from FIG. 13 that there are no pins, or other structures, that project above the top surface of the vacuum chuck 140. This permits the glass slide 130 to be moved in any direction parallel with the top surface without constraint.

Turning now to FIG. 14, a very high numerical aperture illuminator 1400 for an LCM device is depicted. The illuminator 1400 provides a large working distance. A fiber optic 1410 provides a source of white light illumination. The diverging beam 1420 from the fiber optic 1410 can have a numerical aperture of approximately 0.4. A collimator lens 1430 collimates the light from the fiber optic 1410. The collimator lens 1430 can be an aspheric lens (e.g., a Melles

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Griot (01 LAG 025) aspheric-like lens). A collimated beam 1440 from the collimator lens 1430 then passes though a beam splitter 1450. The beam splitter 1450 permits the injection of a laser beam 1460. After reflection by the beam splitter 1450, the laser beam 1460 is coaxial with the white light illumination.

5   Both types of light then reach a condenser lens 1470. Condenser lens 1470 can be a Melles Griot (01 LAG 010) or (01 LAG 010) or other similar aspheric-like lens. The condensed coaxial beams are then incident upon and pass through the microcentrifuge tube cap 120. The focusing beam that results from the condenser lens 1470 can have a numerical aperture of approximately 0.8. This

10   can be characterized as a focusing beam. The microcentrifuge tube cap 120 is located on top of a slide with cells to be sampled (not shown).

Turning now to FIG. 15, another embodiment of the high numerical aperture illuminator is depicted. In this embodiment, a diffuser 1500 is located beneath the condenser lens 1470 at above the glass slide 130 that contains the

15   cells to be sampled.

More generally, any suitable scattering media can be used to provide the functions of the diffuser 1500. Providing such a scattering media near the tissue to scatter the light results in dramatically improved illumination of the sample and much better visualization. A scattering media of this type eliminates the

20   need for refractive index matching of the sample. Such a scattering media can allow visualization of the cell nucleus and other subcellular structures that would normally be obscured by normal illumination techniques.

The scattering media can be a diffuser material. A diffuser material that is suitable for use as the scattering media is milk or opal glass which is a very

25   dense, fine diffuser material. For instance, a suitable milk glass is available from Edmund Scientific as Part No. P43,717. Standard laser printer/photocopier paper can even be used as the scattering media. Other types of transparent scattering media can be used, such as, for example, frosted glass, a lenticular sheet, a volume diffuser, and/or a surface diffuser. In any event, the

30   scattering media should be a material that aggressively scatters the illumination

light. A single sheet of typical ground glass is generally inadequate and needs to be combined in multiple layers as a serial stack of three or four sheets of ground glass to diffuse the illumination light sufficiently.

5   Turning now to FIG. 16, after the diffuser 1500 is replaced with a microcentrifuge tube cap 120, the desired cells can be located using the image acquired during the step represented in FIG. 15. Then, the laser beam 1460 can be introduced, reflected off the beam splitter 1450 and directed into the microcentrifuge tube cap 120 so as to acquire the desired sample.

        The purpose of the illuminator design is to provide a very high
10   numerical aperture illuminator for an LCM device. Such an LCM device requires a large working distance. While an illuminator that uses a 40X objective with 0.8 numerical aperture may seem to give better visualization, this design has problems since the working distance for the 40X objective is very small, (e.g., less than 1 millimeter). Thus it is critical for a design that uses a
15   thick dome carrier to have an illumination design with a much longer working distance. A thick dome carrier is a sample carrier whose top and bottom are spaced apart more than a small distance. This is important because the sample is adjacent the bottom of the sample carrier and the objective cannot move closer to the sample than the top of the sample carrier.

20   The focusing lens 190 can be replaced with a Melles Griot aspheric condenser lens such as a 01 LAG 010. Such a lens has a numerical aperture of about 0.75 and a working distance of about 25 millimeters. Such a lens is not corrected for chromatic aberrations like the 40X objective. Experiments done using a spherical lens as a condenser gave good improvement in visualization.
25   This spherical lens clearly did not have the corrections for aberrations that are built into the 40X objective.

        The laser beam can be focused through this condenser lens like the focusing lens 190. This condenser lens has roughly one-half the focal length of the current lens so the laser beam will be focused down to roughly 15 microns.

In an alternative embodiment the design could use a compound lens like the lens in a barcode scanner. Such a compound lens would have a central region for the laser and a surrounding region that would act as a high numerical aperture with regard to the white light illumination.

5   Turning now to FIG. 17, in one embodiment the diffuser 1500 can be located adjacent to the microcentrifuge tube cap 120. In this embodiment the microcentrifuge tube cap 120 is located just above the glass slide 130. Collimated light 1700 is incident upon the diffuser 1500. The diffuser 1500 causes the collimated light to enter into and pass through the cap at an infinite 10   variety of angles. In this way, shadows are reduced and the quality of the imagery is improved.

The diffuser 1500 can be a volumetric diffuser or a surface diffuser. In the case of a volumetric diffuser, the diffuser 1500 can be frosted glass, a speckle based holographic diffuser or even a piece of paper. In the case of a 15   surface diffuser, the diffuser 1500 can be a lenticular sheet, a speckle based holographic surface diffuser or any other suitable topological surface.

Turning now to FIG. 18, the diffuser 1500 in this embodiment is located adjacent to the bottom of the microcentrifuge tube cap 120. The collimated light 1700 passes through the microcentrifuge tube cap 120 and is incident upon 20   the diffuser 1500. As the previously collimated light emerges from the diffuser 1500 it is scattered into a wide range of angles. In this embodiment, the diffuser 1500 is spaced apart from the glass slide 130.

The scattering media (e.g., diffuser 1500) can be directly or indirectly connected to the transfer film carrier and/or the LCM transfer film. 25   Alternatively, the scattering media can be formed on a surface of, or the interior of, the transfer film carrier and/or the LCM transfer film. The scattering media can be fabricated so as to shape the LCM beam and/or the illumination beam. The scattering media needs to be within a few millimeters of the sample to be effective. A few millimeters means less than one centimeter, preferably less 30   than five millimeters.

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The process of operating the instrument begins by visualizing the tissue from which the sample is to be acquired. The tissue is then moved to bring the portion that is to be acquired directly below the principal axis of the instrument. A laser capture microdissection transfer film is then set over the desired area. In

5   a preferred embodiment the film is spaced to within a few microns of the top surface of the sample. Alternatively, the film can be placed in contact with the top of the sample with a pressure sufficient to allow transfer without forcing nonspecific transfer. Finally, the laser is pulsed to heat the film and remove the tissue. The film needs to be pulled off of the sample quickly. Though the

10   velocity should be such that the sample is thixotropically sheared.

Small Spot Size Laser Capture Microdissection

The invention includes a method for microdissecting small regions of

15   tissue samples. The invention also includes equipment adapted to facilitate laser capture microdissection of small tissue regions. Tissue regions with diameters under approximately 20 microns, approximately 10 microns, approximately 5 microns and/or approximately 1 micron in diameter can be defined as small. Such small tissue regions require small spot sizes to be laser

20   capture microdissected.

In the past, the original method for microdissection using laser capture microdissection (LCM) employed a freestanding film that was applied to the surface of the tissue by gently pressing the film onto the sample. The film above the tissue section of interest was then heated and melted by a several

25   hundred-millisecond (msec.) pulse from a CO_2 laser. The length of the laser pulse was chosen so as to allow the film to reach thermal equilibrium during the laser pulse, melting the film over the laser exposure region, but allowing lower laser powers to be used and minimizing the peak temperature experienced by the tissue, thereby preventing damage to DNA/RNA and/or proteins in the

30   sample. This original method avoided short pulse since there appeared to be no

advantage to shorter pulses for melting the film and the shorter pulses exposed the tissue to higher laser fluences, thereby potentially damaging the sample.

In general, the size of the transferred tissue region is determined by the diameter of the portion of the LCM transfer film that is raised to a temperature at which the LCM transfer film becomes hot enough to melt and fuse with the tissue sample. For long pulses, the transfer size is determined primarily by the power of the laser beam and by the thermal characteristics (e.g., thermal capacity and diffusion) of the LCM transfer film. For pulse widths of from approximately 50 msec. to approximately 250 msec., laser beam power of approximately 50 mW, beam diameter of approximately 50-100 microns, and film thickness of approximately 100 microns, the tissue transfer size is roughly the same size as the laser beam diameter. As the power is increased, the transfer size increases. However, for pulse widths of from approximately 50 msec. to approximately 250 msec., the diameter of the transferred spot does not vary as a function of the pulse duration.

As discussed above with regard to the invention, the LCM transfer film can be attached to a flat, rigid carrier rather than being used as a free standing film. This approach offers several advantages. When the LCM transfer film is mounted on a rigid film carrier, the transfer film is injected into the tissue when the laser heats the transfer film. In more detail, when the transfer film is exposed to the laser beam, the film in the exposed region expands, pushing against the rigid film carrier and against the surrounding unheated portions of the LCM transfer film. This expansion forces the melted transfer film downwards and into the sample tissue. Thus, the LCM transfer film need not be in direct contact with the sample tissue. The heated portion of the transfer film reaches out during the laser pulse, fuses with the tissue, and then the tissue is captured within the perimeter of that portion of the transfer film that is exposed to the laser beam.

With a 40 micron thick film doped with roughly 0.1% by weight infrared absorbing dye (e.g., vanadyl phthalocyanine, Aldrich #CA 105011-00-5)

mounted on a flat plexiglass substrate, and using a laser diode tuned to the peak of the absorption bands of the dye, with a pulse width of 50 msec., a laser power of approximately 30 mW, and a beam size of approximately 30 microns, the transferred tissue region is roughly the same size as the laser beam. Further, for
5   these parameters, the transfer spot size diameter increases as the laser power is increased, but the diameter of the transfer spot size does not increase substantially as the pulse duration (aka pulse width) is increased. Significantly, as the laser spot size is reduced below approximately 20 microns, the transfer size does not get smaller, for a pulse duration of approximately 50 msec., or
10   longer.

        An important aspect of the invention is to provide a method that allows the acquisition of an LCM transfer region of less than approximately 30 microns, preferably less than approximately 20 microns, more preferably less than approximately 10 microns, even less than approximately 5 microns. It has
15   been found that a reliable method for obtaining smaller transfer spot sizes (e.g., less than approximately 10 microns in diameter) includes reducing the pulse width of the laser to less than 5 msec., preferably less than approximately 1 msec., even less than approximately 0.5 msec. and simultaneously increasing the peak power of the laser to at least approximately 50 mW. Significantly,
20   these pulse widths and powers do not damage the macromolecules in tissue samples.

        At longer pulse widths, it is believed that the exposed region of the LCM transfer film reaches thermal equilibrium. In these circumstances the width of the transfer region is defined roughly by the diameter of the film region that has
25   a temperature hot enough at thermal equilibrium to melt the film and fuse the film to the tissue. Since the film region excited by the laser has reached thermal equilibrium with the laser operating in the long pulse width regime, the size of the transfer region is substantially independent of the pulse width of the laser pulse. Moreover, if the laser spot size is reduced below roughly 20 microns, the
30   diameter of the melted region of the film at thermal equilibrium remains

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approximately 20-30 microns. Thus, even at smaller beam diameters, the transfer size remains 20-30 microns since this is the size of the heated region at equilibrium.

5 However, at pulse widths below approximately 1 msec., the film does not reach thermal equilibrium and the film in the region exposed to the laser beam heats up quickly, expanding to contact, and fuse, with the tissue. For a short pulse (e.g., less than approximately 1 msec.) there is insufficient time for the temperature of the film outside the region exposed to the laser pulse to increase since the laser pulse is so short. The region of the film outside the exposed area remains below the melting point and does not fuse to the tissue.
10 Thus, for short pulses the transfer region size is not increased by thermal diffusion. For short pulses, the transfer size is determined primarily by the laser spot diameter. For short pulses, the transfer spot size increases as the pulse width of the laser is increased. This is in contrast to the longer pulse regime, indicating operation in a different thermal diffusion regime.
15

Previous embodiments of LCM (using CO_2 or diode lasers and an LCM transfer film that is not mounted to a film carrier) taught against the use of short pulses since short pulses required an increase in the laser fluence experienced by the tissue sample. It was thought that this fluence should be minimized to
20 minimize tissue damage. Moreover, at spot sizes above approximately 50 microns and film thicknesses of from 50 to 100 microns, no advantage was observed in going to very short pulses.

It is surprising that short pulse durations provide sufficient time for the film to expand and contact the tissue. It is also surprising that short pulse
25 widths allow sufficient time to melt the film and fuse the film to the tissue. The discovery by the inventors that reliable LCM at short pulse widths is even possible is a most surprising result and it is even more surprising that small transfer diameters require short pulse excitation. In general, whenever the diameter of the laser beam becomes substantially smaller than the thickness of
30 the LCM transfer film, it may be important to implement the LCM process

using short pulses in order to avoid thermal diffusion and subsequent broadening of the transfer spot size.

Turning to FIG. 19, two spatial domain energy profiles delivered to the LCM transfer film (e.g., EVA) by two different laser beam diameters are depicted. These energy profiles display energy (E) as a function of perpendicular distance from the optical axis (DIA) of the beam. A 5 micron diameter laser beam energy profile is depicted with a solid line in FIG. 19. A 30 micron diameter laser beam energy profile is depicted with a dashed line in FIG. 19. These two profiles illustrate the comparison of the size of the energy profile delivered to the EVA by the laser and the approximate size of the resultant transfer spot. It can be appreciated that the profiles shown in FIG. 19 are coaxial and symmetric with regard to a spatial position corresponding to the center of the laser beams.

Turning now to FIG. 20, a two dimensional geometric plot of three isotherms within an LCM transfer film 2000 produced by a constant laser beam diameter generated in long pulse made are depicted. In FIG. 20, the melt depth with a 30 micron spot size is shown. The upper horizontal line represents a tissue/film interface 2010. The lower horizontal line represents a cap/film interface 2020. The area inside a given curve represents that portion of the transfer film that is in a viscous (e.g. liquid) state. It can be appreciated that the three profiles shown in FIG. 20 are coaxial and symmetric with regard to a spatial position that corresponds to the center of the laser beam. The innermost profile represents a set of conditions T1 (time), E1 (energy), the middle profile represents conditions T2, E2, and the outermost profile represents conditions T3, E3, where T1<T2<T3 and E1<E2<E3.

The pulses depicted in FIG. 20 are sufficiently long so that heat diffusion is substantially isotropic. Due to this, the transfer spot size change is only weakly dependent on change in the energy delivered. The added energy is provided by holding the power constant and increasing the pulse duration. In

this long pulse regime, the added energy is simply diffused to the outer transfer film material and contributes very little to any change in spot size.

Turning now to FIG. 21, a two dimensional geometric plot of three isotherms within the LCM transfer film 2000 produced by a constant laser beam diameter generated in short pulse mode are depicted. In FIG. 21, the melt depth with a 5 micron beam spot size is shown. Again, the upper horizontal line represents the tissue/film interface 2010 and the lower horizontal line represents the cap/film interface 2020. It can be appreciated that the profiles shown in FIG. 21 are coaxial and symmetric with regard to a spatial position that corresponds to the center of the beam. The innermost profile represents a set of conditions T1 (time), E1 (energy), the middle profile represents another set of conditions T2, E2, and the outermost profile represents yet another set of conditions T3, E3, where T1<T2<T3 and E1=E2=E3. Thus, the power at T1 is greater than the power at T2, and the power at both T1 and T2 are greater than the power at T3.

The cross over point (protrusion) for the T1, E1 curve above the tissue/film interface 2010 shows the transfer spot size under these conditions. It can be appreciated that each curve has the same total energy, but the T1, E1 curve has high enough power and short enough pulse to breach to the tissue/film interface with liquid EVA (defining the transfer spot size). In all the cases shown in FIG. 21, the time duration is sufficiently short so radial thermal diffusion into the rest of the EVA is minimal (i.e., the heat/energy is anisotropically dissipated, preferentially in the axial direction). Curves T2 and T3 have slightly longer pulse durations (and corresponding lower power levels) which permit enough radial heat diffusion so that the transfer film 2000 at the interface 2010 is not heated to its melting point.

Turning now to FIG. 22, a focusing lens subassembly 2200 includes a frame 2210 that is mechanically coupled to a lens 2220. The frame 2210 is slidably coupled to a mount 2230 that is adapted to be rigidly connected to an optics plate (not shown in FIG. 22) of an LCM apparatus, such as, for example,

an inverted microscope. The frame 2210 can be readily moved along one axis with regard to the mount 2230. This relative movement is depicted by the double-headed arrow in FIG. 22. A ramp 2240 is pivotally coupled to the frame 2210. Pivoting the ramp 2240 permits the inclination of the ramp 2240 to be

5   adjusted with regard to the plane defined by the optical axis of the lens 2220. A stop 2250 is in contact with the ramp 2240 and acts to limit the pivoting motion of the ramp 2240 in one direction (i.e., toward the frame 2210). The pivoting motion of the ramp 2240 away from the stop 2250 is depicted in FIG. 22 with a single-headed arrow.

10   Referring now to FIG. 23, the focusing lens subassembly 2200 is shown in mechanical engagement with an optics plate 2310 of an inverted microscope. Specifically, the mount 2230 is bolted to the optics plate 2310. Relative movement between the frame 2210 and the optics plate 2310 is represented in FIG. 23 with a double-headed arrow.

15   Turning now to FIG. 24, a bracket 2410 is mechanically coupled to the optics plate 2310. A pivoting arm 2420 is pivotally connected to the bracket 2410. The pivoting arm 2420 is mechanically coupled to the ramp 2240 via a contact point (not shown in FIG. 24). A handle 2430 is connected to the pivoting arm 2420. The pivotable arm 2420 can be provided with mechanical

20   detents to give tactile feedback to a human operator, who can angularly displaces the handle 2430. A rotatable knob 2440 is mechanically coupled to the pivoting arm 2420. The rotatable knob 2440 permits adjustment of the contact point which moves the ramp 2240, thereby providing a fine focus adjustment for the lens 2220.

25   Still referring to FIG. 24, angular deflection of the handle 2430 causes the pivoting arm 2420 to rotate with regard to the bracket 2410, thereby causing the contact point to be moved along the ramp 2240. Gravity can keep the inclined surface of the ramp 2240 adjacent to the contact point. Thus, movement of the contact point across the inclined plane surface of the ramp

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2240 causes the frame 2210 (and therefore, the lens 2220) to be moved with regard to the optics plate 2310.

Referring now to FIG. 25, the focusing lens subassembly 2200 and the optic plate 2310 are shown form a slightly different perspective so that the

5   contact point 2510 is visible. But more importantly, in FIG. 25, the handle 2430, the pivoting arm 2420, and the rotatable knob 2440 have been angularly deflected with regard to the bracket 2410 compared to their position as depicted in FIG. 24. Referring to FIG. 25, it can be appreciated that the contact point 2510 has been displaced to a different point on inclined surface of the ramp

10   2240, compared to the position of the contact point 2510 in FIG. 24. Specifically, with regard to the frame 2210, the contact point 2510 has been repositioned to a lower point of the ramp 2240 in FIG. 25. The position of the contact point 2510 in FIG. 25 can correspond to a large spot size diameter while the position of the contact point in FIG. 24 can correspond to a small spot size

15   diameter. Of course, an intermediate spot size diameter can be defined by an intermediate position of the pivotable arm 2420 that is between the positions shown in FIG. 24 and FIG. 25.

Operation of the LCM apparatus can be made more convenient and more rapid by providing equipment that automatically adjusts the pulse duration and

20   laser beam power as a function of the position of the pivotable arm 2420. This allows the operator to concentrate on choosing the optimum spot size while the apparatus automatically adjusts the pulse duration and laser beam power.

For example, the position shown in FIG. 24 of the pivotable arm 2420 can correspond to a spot size diameter of from approximately 5 microns to

25   approximately 7 microns. When the apparatus is positioned into the configuration shown in FIG. 24, the pulse width duration can be automatically set to within a range of from approximately 100 microseconds to approximately 2 milliseconds, preferably less than 1 millisecond. Similarly, the power of the beam can be automatically adjusted to a range of from approximately 10

30   milliwatts to approximately 100 milliwatts. These automatic settings can be

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achieved with a multiposition switch that is incorporated into the pivoting arm 2420 in combination with a look-up table stored in a memory. In preferred embodiments, the look-up table can be (re)programed by a human operator, or other outside data source.

5   As another example, the position of the pivotable arm 2420 in FIG. 25 can correspond to a spot size diameter of approximately 30 microns. When the apparatus is positioned into the configuration shown in FIG. 25, the pulse duration can be automatically adjusted to a value of from approximately 20 milliseconds to approximately 60 milliseconds. Similarly, in this position, the

10   power of the laser beam can be automatically adjusted to within a range of from approximately 20 milliwatts to approximately 100 milliwatts.

     In this way, a human operator can select a spot size diameter by angular displacement of the handle 2430 with an easy movement of their left hand, while the pulse duration and power are automatically readjusted. Specific

15   angular positions of the pivotable arm 2420 that result in automatic adjustment of the pulse duration, beam power, and/or other operational parameters of the LCM apparatus can be termed opto-interrupts. Of course, the specific spot size diameters, pulse durations, and beam powers given in the two examples above are merely illustrative. Preferred embodiments of the invention can permit the

20   operator, or other programmer, to redefine the parameter envelopes associated with specific angular positions of the handle 2430, the pivotable arm 2420 and the rotatable knob 2440. The envelope endpoints and other operational parameters can be stored in the memory of a computing device.

25   **Practical Applications of the Invention**

     A practical application of the invention that has value within the technological arts is the collection of a large database of gene expression patterns of both healthy and diseased tissue, at different stages of diseases. This

30   database will be used to more fully understand that pathogenesis of cancer and

infectious diseases. The invention will enable a scientist to identify gene

patterns and incorporate this information into effective diagnostics for disease.

The invention will allow medical doctors to compare actual patient tissue

samples with archived data from patient samples at different disease stages,

5   thereby allowing them to prescribe more effective stage therapies, eliminate

unnecessary procedures, and reduce patient suffering. Other research areas

where the invention will find use are drug discovery, developmental biology,

forensics, botany, and the study of infectious diseases such a drug-resistant

tuberculosis. There are virtually innumerable uses for the invention, all of which

10   need not be detailed here.

Advantages of the Invention

A laser capture microdisection instrument and/or method representing an

15   embodiment of the invention can be cost effective and advantageous for at least

the following reasons. The invention will replace current methods with better

technology that allows for more accurate and reproducible results. The

invention can be used to provide a low cost injection molded polymer

disposable that integrates a laser capture microdissection transfer film into the

20   interior surface of an analysis container such as a microcentrifuge tube (e.g., an

EPPENDORF™ tube).

All publications, patent applications, and issued patents mentioned in

this application are hereby incorporated herein by reference in their entirety to

the same extent as if each individual publication, application, or patent was

25   specifically and individually indicated to be incorporated in its entirety by

reference.

All the disclosed embodiments of the invention described herein can be

realized and practiced without undue experimentation. Although the best mode

of carrying out the invention contemplated by the inventors is disclosed above,

30   practice of the invention is not limited thereto. It will be manifest that various

additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

5   For example, the individual components need not be formed in the disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further,

10   although the LCM instrument disclosed herein is described as a physically separate module, it will be manifest that the LCM instrument may be integrated into other apparatus with which it is associated. Furthermore, all the disclosed elements and features of each disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed

15   embodiment except where such elements or features are mutually exclusive.

It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. It is intended that the scope of the invention as defined by the appended claims and their

20   equivalents cover all such additions, modifications, and rearrangements. The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase "means-for." Expedient embodiments of the invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A laser capture microdissection method, comprising:

5   providing a sample that is to undergo laser capture microdissection;

      positioning said sample within an optical axis of a laser capture microdissection instrument, said laser capture microdissection instrument including an illumination/laser beam delivery system;

      providing a transfer film carrier having a substrate surface and a laser

10 capture microdissection transfer film coupled to said substrate surface;

      placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a

15 remainder of said sample to said laser capture microdisection film; then

      illuminating said sample with said illumination/laser beam delivery system; and then

      transferring a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample

20 to said laser capture microdissection transfer film, by delivering energy to said laser capture microdissection transfer film with a pulse from said illumination/laser beam delivery system, wherein thermal energy from said pulse is anisotropically dissipated along an axial direction that is substantially parallel to said optical axis.

25

2. The laser capture microdissection method of Claim 1, further comprising reducing a spot size defined by said illumination/laser beam delivery system before the step of transferring a portion of said sample to said laser capture microdissection transfer film.

30

3.   The laser capture microdissection method of Claim 2, wherein reducing a spot size includes moving a focusing lens.

4.   The laser capture microdissection method of Claim 3, wherein moving a
5   focusing lens includes moving a contact point along a ramp that is mechanically coupled to said focusing lens.

5.   The laser capture microdissection method of Claim 4, wherein moving a contact point includes rotating a pivotable arm that is mechanically coupled to
10   said contact point.

6.   The laser capture microdissection method of Claim 2, wherein reducing a spot size includes resetting an operational parameter.

15   7.   The laser capture microdissection method of Claim 6, wherein resetting an operational parameter includes resetting a pulse duration of said illumination/laser beam delivery system.

8. ·   The laser capture microdissection method of Claim 6, wherein resetting
20   an operational parameter includes resetting a laser beam power of said illumination/laser beam delivery system.

9.   The laser capture microdissection method of Claim 1, further comprising removing said laser capture microdissection film from said sample, after the
25   step of transferring, by moving both said laser capture microdissection transfer film and said portion of said sample away from said remainder of said sample and out of said optical axis with a pivotable transfer arm.

10.   The laser capture microdissection method of Claim 1, wherein said
30   sample is provided on a slide and said laser capture microdissection instrument

includes a translation stage, and further comprising holding said slide against said translation stage by actuating a vacuum circuit to exert a force between said slide and said translation stage, before the step of transferring said portion of said sample to said laser capture microdissection transfer film.

5

11. A laser capture microdissection instrument, comprising:

an illumination/laser beam delivery system adapted to produce anisotropic dissipation of thermal energy within a transfer film along an optical axis of said illumination/laser beam delivery system.

10

12. The laser capture microdissection instrument of Claim 11, further comprising an automatic adjustment of an operational parameter as a function of a selected spot size.

15   13. The laser capture microdissection instrument of Claim 12, wherein said automatic adjustment of an operational parameter includes automatic adjustment of pulse duration.

14. The laser capture microdissection instrument of Claim 12, wherein said

20   automatic adjustment of an operational parameter includes automatic adjustment of laser beam power.

15. The laser capture microdissection instrument of Claim 11, wherein said illumination/laser beam delivery system includes a variable position focusing

25   lens that is adapted to produce anisotropic dissipation of thermal energy within said transfer film along said optical axis of said illumination/laser beam delivery system.

16. The laser capture microdissection instrument of Claim 15, further

30   comprising a ramp mechanically coupled to said variable position focusing lens.

17.   The laser capture microdissection instrument of Claim 16, further comprising a pivotable arm mechanically coupled to said ramp through a contact point.

5   18.   The laser capture microdissection instrument of Claim 11, wherein said laser capture microdissection instrument is an inverted microscope.

19.   The laser capture microdissection instrument of Claim 18, further comprising a translation stage, including a vacuum circuit adapted to exert a
10   force between said translation stage and a sample holder upon which a sample that is to undergo laser capture microdissection is positioned.

20.   The laser capture microdissection instrument of Claim 18, further comprising a pivotally mounted transfer arm adapted to move laser capture
15   microdissection transfer film carriers into, and out of, said optical axis.

FIG. 1

WHITE LIGHT ILLUMINATION PATH
LASER BEAM PATH

FIG. 2A   **FIG. 2B**

FIG.3

FIG. 4

FIG. 5

110
520
112
145
120
147
124

150

FIG. 6

FIG. 7

FIG. 8

FIG. 9

FIG. 10

FIG. 11

FIG.   12

FIG. 13

FIG. 14

F I G.   15

FIG. 16

FIG. 17

FIG. 18

5 MICRON BEAM

30 MICRON BEAM

BEAM PROFILE

Fig 19

E

DIA

2010

Fig 20

TISSUE/FILM INTERFACE

LONG PULSE

T1,E1

PULSE TIME- T
PULSE ENERGY- E

T2,E2

T1<T2<T3
E1<E2<E3

T3,E3

CAP/FILM INTERFACE

2020

SPOT DIA

2010

TISSUE/FILM INTERFACE

Fig 21

T1,E1

SHORT PULSE

2000

T2,E1

PULSE TIME- T
PULSE ENERGY- E1

T3,E1

T1<T2<T3

CAP/FILM INTERFACE

2020

SPOT DIA

Fig. 22

2230 -

2200 →

2210 —

2250

2220 -

2240

Fig. 23

Fig 24

Fig. 25

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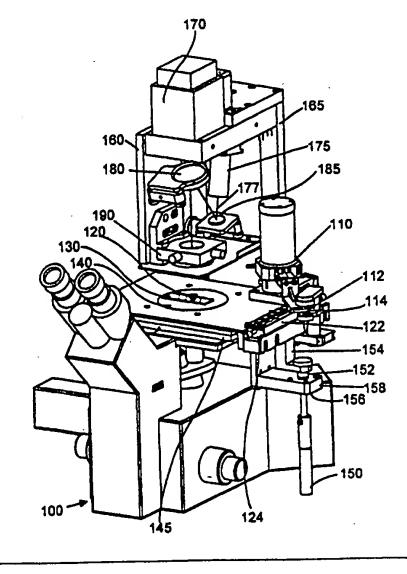
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(54) Title: SMALL DIAMETER LASER CAPTURE MICRODISSECTION

(57) Abstract

Systems and methods for small diameter laser capture microdissection are disclosed. An inverted microscope adapted for small diameter laser capture microdissection includes an illumination/laser beam delivery system with a variable position lens. A method for small diameter laser capture microdissection includes delivering energy having a pulse width of approximately 1 mS, or less, and a peak power of approximately 50 mW, or more. The systems and methods provide the advantage of permitting small regions of tissue samples, for example, under approximately 10 microns in diameter, to be microdissected.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7   G01N1/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 35216 A (BAER THOMAS M ;ENRIGHT MARK A (US); HEAD DAVID F (US); ARCTURUS EN) 13 August 1998 (1998-08-13) claims 2,21	1,11
A	S.R. GOLDSTEIN ET AL.: "Thermal modeling of Laser Capture Microdissection" APPLIED OPTICS., vol. 37, no. 31, 1 November 1998 (1998-11-01), pages 7378-7391, XP002138669 NEW YORK US paragraph '0002!	1,11

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2000	14/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Sarneel, A

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Information on patent family members

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